International Journal of Bio-Technology
and Research (IJBTR)
ISSN (P): 2249-6858; ISSN (E): 2249-796X
Vol. 11 Issue 2, Dec 2021, 37–42

TRANS STELLAR
•Journal Publications •Research Consultancy

TRANSMISSION ELECTRON MICROSCOPIC STUDIES FOR THE IDENTIFICATION OF BACTERIAL ISOLATE FROM RHIZOSPHERE OF BRINJAL (*SOLANUM MELONGENA* L.)

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ABSTRACT

Background:Rhizosphere, phylloplane and caulosphere is the region where a complex community of microbes, mainly bacteria and fungi are present. The microbe plant interaction in these regions can be beneficial, neutral, variable, or deleterious for plant growth. The bacteria that exert beneficial effects on plant development are termed plant growth promoting bacteria. Objectives: To identify the bacteria isolated from rhizosphere of brinjal (Solanum melongena L.) by transmission electron microscope (TEM).Materials and Methods: The pure cultures of bacterial isolate from brinjal (Solanum melongena L.) were used to identify the bacteria. Identification of bacteria was done by transmission electron microscope using reference strain viz., Bacillus polymyxa strain 10401 obtained from France.Results:The BBI revealed rod shaped sporulating bacteria with single polar flagella as observed under transmission electron microscope. The bacterial isolate which showed nitrogen fixing and phosphate solubilizing properties was identified as Bacillus polymyxa belonging to the class Eubacteriales, family Bacillaceae and genera Bacillus. Conclusion: Transmission electron microscope studies revealed that the BBI was a rod shaped bacteria with a single polar flagellum. The bacterial cell measured 2.57 µm (2570 nm) in length and 0.8570 µm (857 nm) in breadth. Furthermore, TEM studies of the sporulating culture revealed the presence of a large central spore, confirming light microscopic observations. The single large spore occupied the entire inner space of the bacterial cell.

KEYWORDS: Brinjal Bacterial isolate (BBI), Transmission Electron Microscopy, Rod Shaped Bacteria & Bacillus Polymyxa

Received: Oct 12, 2021; **Accepted**: Nov 02, 2021; **Published**: Nov 13, 2021; **Paper Id**.: IJBTRDEC20216

Original Article

INTRODUCTION

Brinjal (*Solanum melongena* L.)is a member of the family Solanaceae and a native of India. It is an important vegetable crop of south India. The fruit is rich in vitamin A and vitamin C and isemployed in the Ayurveda system. In Karnataka, brinjal iscultivated in 16,602 hectares of land and has an average yield of 30-35 tons per hectare.The crop needs 369 kg of urea and 80 kg of phosphatic fertilizer per hectare. Although symbiotic nitrogen fixation especially legume-rhizobium system has been proved to be the best form of biological nitrogen fixation, associative nitrogen fixation cannot be ignored. Nitrogen fixation on the rhizoplane, phylloplane and stem have been attributed to the presence of diazotrophic bacteria associated with the roots, stem and leaves of plants.[Dobereiner 1980]

There are many reports relating to the characterisation and identification of the nitrogen fixing bacteria associated with a wide variety of grasses and cereal crops. [Dobereiner 1976,Tarrand 1978,van Berkurn,

1979,1980, Silva et al., 1981; Levanony et al 1987, Rai and Guar, 1988] Hill, 1980; Hill et al., 1983 isolated and characterized rhizosphere bacteria of sweet potato. [Hill et al., 1983] Levanony et al. (1987) identified *Azospirillum brasiliense* in cereal roots using ELISA.Rai and Guar, (1988) characterizedAzotobacterassociated with roots of wheat. Lalande et al. (1989) identified rhizobacteria associated with maize as Azospirillwn. Lalande R (1989) identified Azospirillum brasilense on the surface and endosphere of wheat roots by immunogold labelling. Brand et al. (1991) isolated a root colonizing rhizobacteria, which was characterized as Pseudomonas. Mclnroy and Kloepper (1991) identified endophytic bacte1ia of maize and cotton.

Penot et al., 1991 and Mclnroy and Kloepper, 1991, characterized Azospirillum associated with maize cultivated in France, using biochemical tests. Agarwala-Dutt et al. (1991) isolated associated bacteria from the interiors of many graminaceous plants, many of which were identified as Azospirillum.Lukin (1990) observed spatial distribution of associated bacteria identified as *Azospirillum brasilense* the rhizosphere of barley plants. Holguin et al. (1992) isolated and identified rhizobacteria associated with mangrove trees as staphylococcus. Fages and Mulard (1986) identified the isolated bacteria from the rhizosphere of sunflower as Azospirillwn.

With this background, the present study was designed with the main purpose to identify the brinjal bacterial isolate (BBI) from the rhizosphere of brinjal through transmission electron microscopic (TEM) studies.

MATERIALS AND METHODS

The isolated BBI was subcultured in solid and liquid nitrogen free liquid Burk's media. The pure cultures of BBI were used to characterize the bacteria. Identification of the dominantly associated bacterial isolate of brinjal (*Solanum melongena* L.) was done comparing with a reference strain *viz.*, *Bacillus polymyxa* strain 10401 obtained from France.

To Study the Vegetative Cells of BBI

A 24hr pure culture was diluted in 10 ml of sterile distilled water. After thorough shaking, a drop of this suspension was mounted on a copper grid. A drop of the mixture containing bovine serum albumin (0.1%) and PTA with BSA (Buffered phosphotungstate with bovine serum albumin in the ratio of 1:2 was placed on the support film side of the copper grid. After 30 seconds excess fluid was drained off from the grid by touching its edge to the filter paper leaving a very thin film of liquid.The grid was stained with uranyl acetate before drying. The preparation was examined by transmission electron microscope. Several morphological observations like size, shape, presence of flagella was recorded.

To Study the Sporulating Cells of BBI

A 48hr pure culture of BBI in sporulating media (Brown ME 1968) incubated at 37°C (pH 6.7) was used for the study. The culture was harvested and suspended in physiological saline. One drop of this suspension in four parts of PTA with BSA was mounted on copper grids. After 30 seconds excess fluid was drained off from the grid and stained with uranyl acetate before drying. The preparation was observed under an electron microscope.

RESULTS

To Study the Vegetative Cells of BBI

Transmission electron microscope studies confirmed that the BBI was a rodshaped bacteria with a single polar flagellum (Plate-1). The bacterial cell measured 2.57 µm (2570nm) in length and 0.8570 µm (857nm) in breadth.

To Study the Sporulating Cells of BBI

TEM studies of the sporulating culture revealed the presence of a large central spore, confirming light microscopic observations (Plate-2). The single large spore occupied the entire inner space of the bacterial cell.

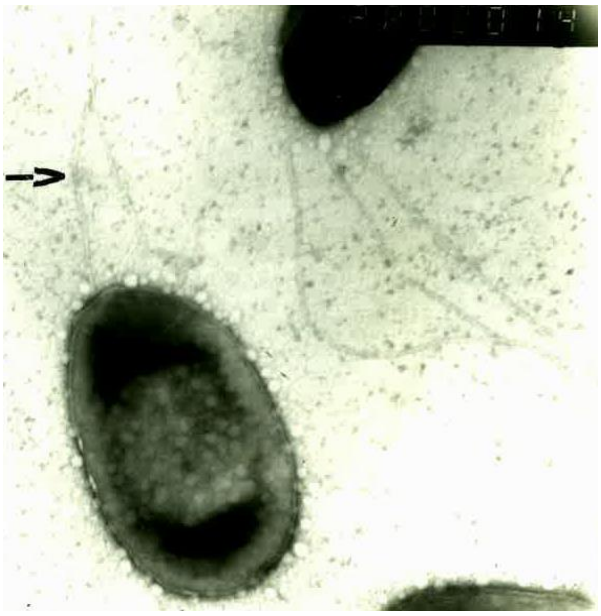

Plate-2: ***Single cell of BBI Observed under Transmission Electron Microscope showing the Presence of Polar Flagella*** **(1000x)**

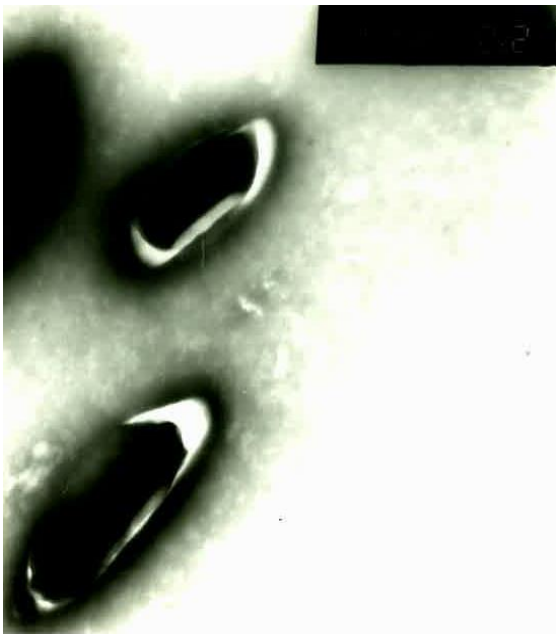

Plate-2: ***Sporulating cell of BBI Observed under Transmission Electron Microscopy*** **(1400x)**

DISCUSSIONS

The bacterial isolate which showed nitrogen fixing and phosphate solubilizing properties was identified as *Bacillus polymyxa* belonging to the class Eubacteriales, family Bacillaceae and genera Bacillus (Bergey's Manual of determinative bacteriology 8th edition), which includes another phosphate solubilisers*viz*., *Bacillus macerans*. *Bacillus polymyxa* appeared to be rod shaped with single polar flagellum with spore observed under a transmission electron microscope.Growth promoting properties of *Bacillus polymyxa* have been reported by Holl andChanway (1992) in pine seedlings. These studies further support the present finding of growth promotion by *Bacillus polymyxa* isolated from the roots of brinjal (*Solanum melongena* L.).The single spore covered the entire space of the cell. The vegetative cell measured 2570µm x 0.8570 µm and had single long polar flagella that helped in movement and infibrillar attachment.These bacteria are gram +ve, Shawky and Sawaby (1991), Silva et al (1981) and Umali-Gracia (1981) did similar identification of the isolated bacteriausing electron microscopy. Shawky and Sawaby (1991) identified the isolate as Azotobacter using transmission electron microscopy based on external morphology, sporulation and germination. Levanony et al (1987) used ELISA for specific identification of the rhizobacteria isolated from cereal roots, which was characterized as Azospirillum. Growth promoting properties of *Bacillus polymyxa* have been reported by Holl andChanway (1992), in pine seedlings. These studies further support the present finding of growth promotion by *Bacillus polymyxa* isolated from the roots of brinjal (*Solanum melongena* L.).

CONCLUSIONS

Transmission electron microscope studies revealed that the BBI was a rod shaped bacteria with a single polar flagellum. The bacterial cell measured 2.57 µm (2570 nm) in length and 0.8570 µm (857 nm) in breadth. Furthermore, TEM studies of the sporulating culture revealed the presence of a large central spore, confirming light microscopic observations. The single large spore occupied the entire inner space of the bacterial cell.

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